

# UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

## APPLICATION ELEMENTS

*See MPEP chapter 600 concerning utility patent application contents.*

1.  \*Fee Transmittal Form (e.g., PTO/SB/17)  
(Submit an original and a duplicate for fee processing)

2.  Specification (preferred arrangement set forth below)

Total Pages

29

- Descriptive title of the Invention
- Cross References to Related Applications
- Reference of Microfiche Appendix
- Background of the Invention
- Brief Summary of the Invention
- Brief Description of the Drawings (if filed)

- Detailed Description

- Claim(s)

- Abstract of the Disclosure

3.  Drawing(s) (35 U.S.C. 113)

Total Sheets

[ ]

4.  Oath or Declaration

Total Pages

[ ]

- a.  Newly executed (original or copy)

- b.  Copy from a prior application (37 C.F.R. § 1.63(d))  
(for continuation/divisional with Box 17 completed)

i.  DELETION OF INVENTOR(S)

Signed statement attached deleting inventor(s)  
named in the prior application, see 37 C.F.R. §§  
1.63(d)(2) and 1.33 (b)

5.  Incorporation By Reference (useable if Box 4b is checked)

- The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered to be a part of the disclosure of the accompanying application and is hereby incorporated by reference thereto.

17. If a CONTINUING APPLICATION, check appropriate box, and supply the requisite information below and in a preliminary amendment:

- Continuation     Divisional

- Continuation-in-part (CIP)

of prior application No:

Prior application information:

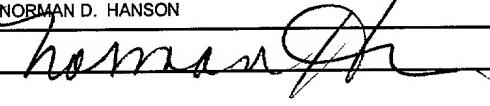
Examiner:

Group / Art Unit:

## 18. CORRESPONDENCE ADDRESS

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			11/30/1999

ADDRESS TO: Assistant Commissioner for Patents  
Box Patent Application  
Washington, DC 20231

6.  Microfiche Computer Program (Appendix)

7. Nucleotide and/or Amino Acid Sequence Submission  
(if applicable, all necessary)

- a.  Computer Readable Copy
- b.  Paper Copy (identical to computer copy)
- c.  Statement verifying identity of above copies

JC 678 U.S. PTO  
09/451739  
11/30/99

## ACCOMPANYING APPLICATION PARTS

8.  Assignment Papers (cover sheet & document(s))

9.  37 C.F.R. § 3.73(b) Statement  
(when there is an assignee)  Power of Attorney

10.  English Translation Document (if applicable)

11.  Information Disclosure Statement (IDS)/PTO-1449  Copies of IDS Citations

12.  Preliminary Amendment

13.  Return Receipt Postcard (MPEP 503)  
(Should be specifically itemized)

14.  \*Small Entity Statement(s)  Statement filed in prior application,  
(PTO/SB/09-12) Status is proper and desired

15.  Certified Copy of Priority Document(s)

16.  Other:

\*NOTE FOR ITEMS 1 & 14: IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY FEES, A SMALL ENTITY STATEMENT IS REQUIRED (37 C.F.R. § 1.27), EXCEPT IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37 C.F.R. § 1.28)

ISOLATED NUCLEIC ACID MOLECULES ENCODING  
CANCER ASSOCIATED ANTIGENS, THE ANTIGENS PER SE,  
AND USES THEREOF

## FIELD OF THE INVENTION

This invention relates to antigens associated with cancer, the nucleic acid molecules encoding them, as well as the uses of these.

## BACKGROUND AND PRIOR ART

It is fairly well established that many pathological conditions, such as infections, cancer, autoimmune disorders, etc., are characterized by the inappropriate expression of certain molecules. These molecules thus serve as "markers" for a particular pathological or abnormal condition. Apart from their use as diagnostic "targets", i.e., materials to be identified to diagnose these abnormal conditions, the molecules serve as reagents which can be used to generate diagnostic and/or therapeutic agents. A by no means limiting example of this is the use of cancer markers to produce antibodies specific to a particular marker. Yet another non-limiting example is the use of a peptide which complexes with an MHC molecule, to generate cytolytic T cells against abnormal cells.

Preparation of such materials, of course, presupposes a source of the reagents used to generate these. Purification from cells is one laborious, far from sure method of doing so. Another preferred method is the isolation of nucleic acid molecules which encode a particular marker, followed by the use of the isolated encoding molecule to express the desired molecule.

Two basic strategies have been employed for the detection of such antigens, in e.g., human tumors. These will be referred to as the genetic approach and the biochemical approach. The genetic approach is exemplified by, e.g., dePlaen et al., Proc. Natl. Sci. USA 85: 2275 (1988), incorporated by reference. In this approach, several hundred pools of plasmids of a cDNA library obtained from a tumor are transfected into recipient cells, such as COS cells, or into antigen-negative variants of tumor cell lines which are tested for the expression of the

specific antigen. The biochemical approach, exemplified by, e.g., O. Mandelboim, et al., Nature 369: 69 (1994) incorporated by reference, is based on acidic elution of peptides which have bound to MHC-class I molecules of tumor cells, followed by reversed-phase high performance liquid chromatography (HPLC). Antigenic peptides are identified after they bind to empty MHC-class I molecules of mutant cell lines, defective in antigen processing, and induce specific reactions with cytotoxic T-lymphocytes. These reactions include induction of CTL proliferation, TNF release, and lysis of target cells, measurable in an MTT assay, or a <sup>51</sup>Cr release assay.

These two approaches to the molecular definition of antigens have the following disadvantages: first, they are enormously cumbersome, time-consuming and expensive; and second, they depend on the establishment of cytotoxic T cell lines (CTLs) with predefined specificity.

The problems inherent to the two known approaches for the identification and molecular definition of antigens is best demonstrated by the fact that both methods have, so far, succeeded in defining only very few new antigens in human tumors. See, e.g., van der Bruggen et al., Science 254: 1643-1647 (1991); Brichard et al., J. Exp. Med. 178: 489-495 (1993); Coulie, et al., J. Exp. Med. 180: 35-42 (1994); Kawakami, et al., Proc. Natl. Acad. Sci. USA 91: 3515-3519 (1994).

Further, the methodologies described rely on the availability of established, permanent cell lines of the cancer type under consideration. It is very difficult to establish cell lines from certain cancer types, as is shown by, e.g., Oettgen, et al., Immunol. Allerg. Clin. North. Am. 10: 607-637 (1990). It is also known that some epithelial cell type cancers are poorly susceptible to CTLs in vitro, precluding routine analysis. These problems have stimulated the art to develop additional methodologies for identifying cancer associated antigens.

One key methodology is described by Sahin, et al., Proc. Natl. Acad. Sci. USA 92: 11810-11913 (1995), incorporated by reference. Also, see U.S. Patent No. 5,698,396, and Application Serial No. 08/479,328, filed on June 7, 1995 and January 3, 1996, respectively. All three of these references are incorporated by reference. To summarize, the method involves the expression of cDNA libraries in a prokaryotic host. (The libraries are secured from a tumor sample). The expressed libraries are then immunoscreened with absorbed and diluted sera, in order to detect those antigens which elicit high titer humoral responses. This methodology is known as the SEREX method ("Serological identification of antigens by Recombinant Expression Cloning"). The methodology has been employed to confirm expression of previously identified tumor associated antigens, as well as to detect new ones. See the above referenced patent applications and Sahin, et al., supra, as well as Crew, et al., EMBO J 14: 2333-2340 (1995).

This methodology has been applied to a range of tumor types, including those described by Sahin et al., supra, and Pfreundschuh, supra, as well as to esophageal cancer (Chen et al., Proc. Natl. Acad. Sci. USA 94: 1914-1918 (1997)); lung cancer (Güre et al., Cancer Res. 58: 1034-1041 (1998)); colon cancer (Serial No. 08/948, 705 filed October 10, 1997) incorporated by reference, and so forth. Among the antigens identified via SEREX are the SSX2 molecule (Sahin et al., Proc. Natl. Acad. Sci. USA 92: 11810-11813 (1995); Tureci et al., Cancer Res. 56: 4766-4772 (1996); NY-ESO-1 Chen, et al., Proc. Natl. Acad. Sci. USA 94: 1914-1918 (1997); and SCP1 (Serial No. 08/892,705 filed July 15, 1997) incorporated by reference. Analysis of SEREX identified antigens has shown overlap between SEREX defined and CTL defined antigens. MAGE-1, tyrosinase, and NY-ESO-1 have all been shown to be recognized by patient antibodies as well as CTLs, showing that humoral and cell mediated responses do act in concert.

It is clear from this summary that identification of relevant antigens via SEREX is a desirable aim. The inventors have applied this methodology and have identified several new antigens associated with cancer, as detailed in the description which follows.

#### DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

##### **EXAMPLE 1**

The SEREX methodology, as described by, e.g. Sahin, et al., Proc. Natl. Acad. Sci. USA 92: 11810-11813 (1995); Chen, et al., Proc. Natl. Acad. Sci. USA 94: 1914-1918 (1997), and U.S. Patent No. 5,698,396, all of which are incorporated by reference. In brief, total RNA was extracted from a sample of a cutaneous metastasis of a breast cancer patient (referred to as "BR11" hereafter), using standard CsCl guanidine thiocyanate gradient methodologies. A cDNA library was then prepared, using commercially available kits designed for this purpose. Following the SEREX methodology referred to supra, this cDNA expression library was amplified, and screened with either autologous BR11 serum which had been diluted to 1:200, or with allogeneic, pooled serum, obtained from 7 different breast cancer patients, which had been diluted to 1:1000. To carry out the screen, serum samples were first diluted to 1:10, and then preabsorbed with lysates of E. coli that had been transfected with naked vector, and the serum samples were then diluted to the levels described supra. The final dilutions were incubated overnight at room temperature with nitrocellulose membranes containing phage plaques, at a density of 4-5000 plaque forming units ("pfus") per 130 mm plate.

Nitrocellulose filters were washed, and incubated with alkaline phosphatase conjugated, goat anti-human Fcγ secondary antibodies, and reactive phage plaques were visualized via incubation with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium.

This procedure was also carried out on a normal testicular cDNA library, using a 1:200 serum dilution.

A total of  $1.12 \times 10^6$  pfus were screened in the breast cancer cDNA library, and 38 positive clones were identified. With respect to the testicular library,  $4 \times 10^5$  pfus were screened, and 28 positive clones were identified.

Additionally,  $8 \times 10^5$  pfus from the BR11 cDNA library were screened using the pooled serum described. Of these, 23 were positive.

The positive clones were subcloned, purified, and excised to forms suitable for insertion in plasmids. Following amplification of the plasmids, DNA inserts were evaluated via restriction mapping (EcoRI-XbaI), and clones which represented different cDNA inserts were sequenced using standard methodologies.

If sequences were identical to sequences found in GenBank, they were classified as known genes, while sequences which shared identity only with ESTs, or were identical to nothing in these data bases, were designated as unknown genes. Of the clones from the breast cancer library which were positive with autologous serum, 3 were unknown genes. Of the remaining 35, 15 were identical to either NY-ESO-1, or SSX2, two known members of the CT antigen family described supra, while the remaining clones corresponded to 14 known genes. Of the testicular library, 12 of the clones were SSX2.

The NY-ESO-1 antigen was not found, probably because the commercial library that was used had been size fractionated to have an average length of 1.5 kilobases, which is larger than full length NY-ESO-1 cDNA which is about 750 base pairs long.

With respect to the screening carried out with pooled, allogeneic sera, four of the clones were NY-ESO-1. No other CT antigens were identified. With the exception of NY-ESO-1, all of the genes identified were expressed universally in normal tissue.

A full listing of the isolated genes, and their frequency of occurrence follows, in tables 1, 2 and 3. Two genes were found in both the BR11 and testicular libraries, i.e., poly (ADP-ribose) polymerase, and tumor suppression gene ING1. The poly (ADP-ribose) polymerase gene has also been found in colon cancer libraries screened via SEREX, as is disclosed by Scanlan, et al., Int. J. Cancer 76: 652-58 (1998) when the genes identified in the screening of the BR11 cDNA library by autologous and allogeneic sera were compared, NY-ESO-1 and human keratin.

**Table 1. SEREX-defined genes identified by autologous screening of BR11 cDNA library**

Gene group	No. of clones	Comments	Expression
CT genes	10	NY-ESO-1	tumor, testis
	5	SSX2	tumor, testis
Non-CT genes	5	Nuclear Receptor Co-Repressor	ubiquitous
	4	Poly(ADP-ribose) polymerase	ubiquitous
	2	Adenylosuccinatelyase	ubiquitous
	2	cosmid 313 (human)	ESTs: muscle, brain, breast
	1	CD 151 (transmembrane protein)	ubiquitous
	1	Human HRY Gen	RT-PCR: multiple normal tissues
	1	Alanyl-t-RNA-Synthetase	ubiquitous
	1	NAD(+) ADP-Ribosyltransferase	ubiquitous
	1	Human keratin 10	ESTs: multiple normal tissues
	1	Human EGFR kinase substrate	ubiquitous
	1	<i>ING 1</i> Tumor suppressor gene	RT-PCR: multiple normal tissues
	1	Unknown gene, NCI_CGAP_Pr12 cDNA clone	ESTs: pancreas, liver, spleen, uterus
	1	Unknown gene	ESTs: multiple normal tissues
	1	Unknown gene	RT-PCR: multiple normal tissues

**Table 2. SEREX-defined genes identified by allogeneic screening of BR11 cDNA library**

Gene group	No. of clones	Comments	Expression
CT genes	4	NY-ESO-1	tumor, testis
Non-CT genes	6	zinc-finger helicase	ESTs: brain, fetal heart, total fetus
	4	Acetoacetyl-CoA-thiolase	ubiquitous
	3	KIAA0330 gene	ESTs: multiple normal tissues
	2	U1snRNP	ubiquitous
	1	Human aldolase A	ubiquitous
	1	Retinoblastoma binding protein 6	ESTs: tonsils, fetal brain, endothelial cells, brain
	1	$\alpha$ 2-Macroglobulin receptor associated protein	ubiquitous
	1	Human Keratin 10	ESTs: multiple normal tissues

**Table 3. SEREX-defined genes identified by screening of a testicular cDNA library with BR11 serum**

Gene group	No. of clones	Comments	Expression
CT genes:	12	SSX2	tumor, testis
Non-CT genes:	3	Rho-associated coiled-coil forming protein	ubiquitous
	3	Poly(ADP-ribose) polymerase	ubiquitous
	3	Gene from HeLa cell, similar to TITIN	ubiquitous
	2	Gene from parathyroid tumor	RT-PCR: multiple normal tissues
	1	Transcription termination factor I-interacting peptide 21	ubiquitous
	1	Gene from fetal heart	ESTs: multiple normal tissues
	1	<i>ING 1</i> tumor suppressor gene	RT-PCR: multiple normal tissues
	1	KIAA0647 cDNA	ESTs: multiple normal tissues
	1	KIAA0667 cDNA	ESTs: multiple normal tissues

**EXAMPLE 2**

The mRNA expression pattern of the cDNAs identified in example 1, in both normal and malignant tissues, was studied. To do this, gene specific oligonucleotide primers were designed which would amplify cDNA segments 300-600 base pairs in length, using a primer melting temperature of 65-70° C. The primers used for amplifying MAGE-1,2,3 and 4, BAGE, NY-ESO-1, SCP1, and SSX1, 2, 3, 4 and 5 were known primers, or were based on published sequences. See Chen, et al. supra; Tureci, et al., Proc. Natl. Acad. Sci. USA 95: 5211-16 (1998). Gure, et al., Int. J. Cancer 72: 965-71 (1997); Chen, et al., Proc. Natl. Acad. Sci. USA 91: 1004-1008 (1994); Gaugler, et al., J. Exp. Med. 179: 921-930 (1994), dePlaen, et al., Immunogenetics 40: 360-369 (1994), all of which are incorporated by reference. RT-PCR was carried out for 35 amplification cycles, at an annealing temperature of 60° C. Using this RT-PCR assay, the breast cancer tumor specimen was positive for a broad range of CT antigens, including MAGE-1,3 AND 4, BAGE, SSX2, NY-ESO-1 and CT7. The known CT antigens SCP-1, SSX1, 4 and 5 were not found to be expressed.

An additional set of experiments were carried out, in which the seroreactivity of patient sera against tumor antigens was tested. Specially, ELISAs were carried out, in accordance with Stockert, et al., J. Exp. Med. 187: 1349-1354 (1998), incorporated by reference, to determine if antibodies were present in the patient sera. Assays were run for MAGE-1, MAGE-3, NY-ESO-1, and SSX2. The ELISAs were positive for NY-ESO-1 and SSX2, but not the two MAGE antigens.

### EXAMPLE 3

Two clones (one from the breast cancer cDNA library and one from the testicular library), were identified as a gene referred to as ING1, which is a tumor suppressor gene candidate. See Garkavtsev, et al., Nature 391: 295-8 (1998), incorporated by reference. The sequence found in the breast cancer library, differed from the known sequence of ING1 at six residues, i.e., positions 818, 836, 855, 861, 866 and 874. The sequence with the six variants is set forth at SEQ ID NO: 1. The sequence of wild type ING1 is set out at SEQ ID NO: 2.

To determine if any of these differences represented a mutation in tumors, a short, PCR fragment which contained the six positions referred to supra was amplified from a panel of allogeneic normal tissue, subcloned, amplified, and sequenced following standard methods.

The results indicated that the sequences in the allogeneic tissues were identical to what was found in tumors, ruling out the hypothesis that the sequence differences were a tumor associated mutation. This conclusion was confirmed, using the testicular library clone, and using restriction analysis of ING1 cDNA taken from normal tissues. One must conclude, therefore, that the sequence information provided by Garkavtsev, et al., supra, is correct.

### EXAMPLE 4

Additional experiments were carried out to determine whether genetic variations might exist in the 5' portion of the ING1 gene, which might differ from the 5' portion of the clone discussed supra (SEQ ID NO: 1). In a first group of experiments, attempts were made to obtain full length ING1 cDNA from both the breast tumor library, and the testicular library. SEQ ID NO: 1 was used as a probe of the library, using standard methods.

Four clones were isolated from the testicular library and none were isolated from the breast cancer library. The four clones, following sequencing, were found to derive from three transcript variants. The three variants were identical from position 586 down to their 3' end, but differed in their 5' regions, suggesting alternatively spliced variants, involving the same exon-intron junction. All three differed from the sequence of ING1 described by Garkavtsev, et al., in Nat. Genet. 14: 415-420 (1996). These three variants are set out as SEQ ID NOS: 1, 3 and 4.

All of the sequences were then analyzed. The ORFs of SEQ ID NOS: 2, 1 and 4 (SEQ ID NO: 2 is the originally disclosed, ING1 sequence), encode polypeptides of 294, 279 and 235 amino acids, of which 233 are encoded by the 3' region common to the three sequences. These putative sequences are set out as SEQ ID NOS: 5, 6 and 7. With respect to SEQ ID NO: 3, however, no translational initiation site could be identified in its 5' region.

## EXAMPLE 5

The data regarding SEQ ID NO: 3, described supra, suggested further experiments to find additional ORFs in the 5-end of variant transcripts of the molecule. In order to determine this, 5'-RACE -PCR was carried out using gene specific and adapted specific primers, together with commercially available products, and standard methodologies.

The primers used for these experiments were:

CACACAGGATCCATGTTGAGTCCTGCCAACGG

CGTGGTCGTGGTTGCTGGACGCG

(SEQ ID NOS: 9 and 10), for SEQ ID NO: 1;

CCCAGCGGCCCTGACGCTGTC

CGTGGTCGTGGTTGCTGGACGCG

(SEQ ID NOS: 11 and 12), for SEQ ID NO: 3; and

GGAAGAGATAAGGCCTAGGGAAG

CGTGGTCGTGGTTGCTGGACGCG

(SEQ ID NOS: 13 and 14), for SEQ ID NO: 4.

Cloning and sequencing of the products of RACE PCR showed that the variant sequence of SEQ ID NO: 5 was 5' to SEQ ID NO: 3, and that full length cDNA for the variant SEQ ID NO: 3 contained an additional exon 609 nucleotides long, positioned between SEQ ID NO: 3 and the shared, 3' sequence referred to supra. This exon did not include an ORF. The first available initiation site would be an initial methionine at amino acid 70 of SEQ ID NO: 1. Thus, if expressed, SEQ ID NO: 3 would correspond to a molecule with a 681 base pair, untranslated 5' end and a region encoding 210 amino acids.

## **EXAMPLE 6**

The presence of transcript variants with at least 3 different transcriptional initiation sites, and possibly different promoters, suggested that mRNA expression might be under different, tissue specific regulation.

To determine this, variant-specific primers were synthesized, and RT-PCR was carried out on a panel of tissues, using standard methods.

SEQ ID NO: 1 was found to be expressed universally in all of the normal breast, brain and testis tissues examined, in six breast cancer lines, and 8 melanoma cell lines, and in cultured melanocytes. SEQ ID NO: 3 was found to be expressed in four of the six breast cancer lines, normal testis, liver, kidney, colon and brain. SEQ ID NO: 4 was only found to be expressed by normal testis cells and weakly in brain cells.

**EXAMPLE 7**

A further set of experiments were carried out to determine if antibodies against ING1 were present in sera of normal and cancer patients. A phase plaque immuno assay of the type described supra was carried out, using clones of SEQ ID NO: 1 as target. Of 14 allogeneic sera taken from breast cancer patients, two were positive at 1:200 dilutions. All normal sera were negative.

**EXAMPLE 8**

The BR11 cDNA library described supra was then screened, using SEQ ID NO: 1 and standard methodologies. A 593 base pair cDNA was identified, which was different from any sequences in the data banks consulted. The sequence of this cDNA molecule is set out at SEQ ID NO: 8.

The cDNA molecule set forth as SEQ ID NO:1 was then used in Southern blotting experiments. In brief, genomic DNA was isolated from normal human tissue, digested with BamHI or Hind III, and then separated onto 0.7% agarose gel, blotted onto nitrocellulose filters, and hybridized using <sup>32</sup>P labelled SEQ ID NO: 1, at high stringency conditions (aqueous buffer, 65 °C). The probes were permitted to hybridize overnight, and then exposed for autoradiography. Two hybridizing DNA species were identified, i.e., SEQ ID NOS: 1 and 8.

**EXAMPLE 9**

The cDNA molecule set forth in SEQ ID NO: 8 was then analyzed. 5'-RACE PCR was carried out using normal fetus cDNA. Full length cDNA for the molecule is 771 base pairs long, without the poly A tail. It shows strong homology to SEQ ID NO: 1, with the strongest

homology in the 5' two-thirds (76% identity over nucleotide 1-480); however, the longest ORF is only 129 base pairs, and would encode a poly peptide 42 amino acids long which was homologous to, but much shorter than, the expected expression product of SEQ ID NO: 1.

In addition to the coding region, SEQ ID NO: 8 contains 203 base pairs of 5'-untranslated region, and 439 base pairs of 3'-untranslated region.

RT-PCR assays were carried out, as described supra. All of the normal tissues tested, including brain, colon, testis, tissue and breast, were positive for expression of this gene. Eight melanoma cell lines were tested, of which seven showed varying levels of expression, and one showed no expression. Six breast cancer cell lines were tested, of which four showed various levels of expression, and two showed no expression.

#### **EXAMPLE 10**

An additional breast cancer cDNA library, referred to as "BR17-128", was screened, using autologous sera. A cDNA molecule was identified.

Analysis of the sequence suggested that it was incomplete at the 5' end. To extend the sequence, a testicular cDNA library was screened with a nucleotide probe based upon the partial sequence identified in the breast cancer library. An additional 1200 base pairs were identified following these screenings. The 2011 base pairs of information are set forth in SEQ ID NO: 15.

The longest open reading frame is 1539 base pairs, corresponding to a protein of about 59.15 kilodaltons. The deduced sequence is set forth at SEQ ID NO: 16.

RT-PCR was then carried out using the following primers:

CACACAGGATCCATGCAGGCCCGCACAGGAG  
CACACAAAGCTCTAGGATTGGCACAGCCAGAG

(SEQ ID NOS: 17 and 18)

Strong signals were observed in normal testis and breast tissue, and weak expression was observed in placenta.

No expression was found in normal brain, kidney, liver, colon, adrenal, fetal brain, lung, pancreas, prostate, thymus, uterus, and ovary tissue of tumor cell lines tested, 2 of the breast cancer lines were strongly positive and two were weakly positive. Of melanoma two of 8 were strongly positive, and 3 were weakly positive. Of lung cancer cell lines, 4 of 15 were strongly positive, and 3 were weakly positive.

When cancer tissue specimens were tested, 16 of 25 breast cancer samples were strongly positive, and 3 additional samples were weakly positive. Two of 36 melanoma samples were positive (one strong, one weak). All other cancer tissue samples were negative.

When Northern blotting was carried out, a high molecular weight smear was observed in testis, but in no other tissues tested.

The foregoing examples describe the isolation of a nucleic acid molecule which encodes a cancer associated antigen. "Associated" is used herein because while it is clear that the relevant molecule was expressed by several types of cancer, other cancers, not screened herein, may also express the antigen.

The invention relates to nucleic acid molecules which encode the antigens encoded by, e.g., SEQ ID NOS: 1, 3, 8, and 15, as well as the antigens encoded thereby, such as the proteins with the amino acid sequences of SEQ ID NOS: 5, 6, 7, and 16. It is to be understood that all sequences which encode the recited antigen are a part of the invention.

Also a part of the invention are expression vectors which incorporate the nucleic acid molecules of the invention, in operable linkage (i.e., "operably linked") to a promoter.

Construction of such vectors, such as viral (e.g., adenovirus or Vaccinia virus) or attenuated viral vectors is well within the skill of the art, as is the transformation or transfection of cells, to produce eukaryotic cell lines, or prokaryotic cell strains which encode the molecule of interest. Exemplary of the host cells which can be employed in this fashion are COS cells, CHO cells, yeast cells, insect cells (e.g., Spodoptera frugiperda), NIH 3T3 cells, and so forth. Prokaryotic cells, such as E. coli and other bacteria may also be used. Any of these cells can also be transformed or transfected with further nucleic acid molecules, such as those encoding cytokines, e.g., interleukins such as IL-2, 4, 6, or 12 or HLA or MHC molecules.

Also a part of the invention are the antigens described herein, both in original form and in any different post translational modified forms. The molecules are large enough to be antigenic without any posttranslational modification, and hence are useful as immunogens, when combined with an adjuvant (or without it), in both precursor and post-translationally modified forms. Antibodies produced using these antigens, both poly and monoclonal, are also a part of the invention as well as hybridomas which make monoclonal antibodies to the antigens. The whole protein can be used therapeutically, or in portions, as discussed infra. Also a part of the invention are antibodies against this antigen, be these polyclonal, monoclonal, reactive fragments, such as Fab, (F(ab)<sub>2</sub>)' and other fragments, as well as chimeras, humanized antibodies, recombinantly produced antibodies, and so forth.

As is clear from the disclosure, one may use the proteins and nucleic acid molecules of the invention diagnostically. The SEREX methodology discussed herein is premised on an immune response to a pathology associated antigen. Hence, one may assay for the relevant pathology via, e.g., testing a body fluid sample of a subject, such as serum, for reactivity with the antigen per se. Reactivity would be deemed indicative of possible presence of the pathology.

So, too, could one assay for the expression of any of the antigens via any of the standard nucleic acid hybridization assays which are well known to the art, and need not be elaborated upon herein. One could assay for antibodies against the subject molecules, using standard immunoassays as well.

Analysis of SEQ ID NO: 1, 3, 4, 8, and 15 will show that there are 5' and 3' non-coding regions presented therein. The invention relates to those isolated nucleic acid molecules which contain at least the coding segment, and which may contain any or all of the non-coding 5' and 3' portions.

Also a part of the invention are portions of the relevant nucleic acid molecules which can be used, for example, as oligonucleotide primers and/or probes, such as one or more of SEQ ID NOS: 9, 10, 11, 12, 13, 14, 17 and 18, as well as amplification product like nucleic acid molecules comprising at least nucleotides 305-748 of SEQ ID NO: 1.

As was discussed supra, study of other members of the “CT” family reveals that these are also processed to peptides which provoke lysis by cytolytic T cells. There has been a great deal of work on motifs for various MHC or HLA molecules, which is applicable here. Hence, a further aspect of the invention is a therapeutic method, wherein one or more peptides derived from the antigens of the invention which bind to an HLA molecule on the surface of a patient's tumor cells are administered to the patient, in an amount sufficient for the peptides to bind to the MHC/HLA molecules, and provoke lysis by T cells. Any combination of peptides may be used. These peptides, which may be used alone or in combination, as well as the entire protein or immunoreactive portions thereof, may be administered to a subject in need thereof, using any of the standard types of administration, such as intravenous, intradermal, subcutaneous, oral, rectal, and transdermal administration. Standard pharmaceutical carriers, adjuvants, such as saponins,

GM-CSF, and interleukins and so forth may also be used. Further, these peptides and proteins may be formulated into vaccines with the listed material, as may dendritic cells, or other cells which present relevant MHC/peptide complexes.

Similarly, the invention contemplates therapies wherein nucleic acid molecules which encode the proteins of the invention, one or more or peptides which are derived from these proteins are incorporated into a vector, such as a Vaccinia or adenovirus based vector, to render it transfectable into eukaryotic cells, such as human cells. Similarly, nucleic acid molecules which encode one or more of the peptides may be incorporated into these vectors, which are then the major constituent of nucleic acid bases therapies.

Any of these assays can also be used in progression/regression studies. One can monitor the course of abnormality involving expression of these antigens simply by monitoring levels of the protein, its expression, antibodies against it and so forth using any or all of the methods set forth supra.

It should be clear that these methodologies may also be used to track the efficacy of a therapeutic regime. Essentially, one can take a baseline value for a protein of interest using any of the assays discussed supra, administer a given therapeutic agent, and then monitor levels of the protein thereafter, observing changes in antigen levels as indicia of the efficacy of the regime.

As was indicated supra, the invention involves, inter alia, the recognition of an “integrated” immune response to the molecules of the invention. One ramification of this is the ability to monitor the course of cancer therapy. In this method, which is a part of the invention, a subject in need of the therapy receives a vaccination of a type described herein. Such a vaccination results, e.g., in a T cell response against cells presenting HLA/peptide complexes on their cells. The response also includes an antibody response, possibly a result of the release of

antibody provoking proteins via the lysis of cells by the T cells. Hence, one can monitor the effect of a vaccine, by monitoring an antibody response. As is indicated, supra, an increase in antibody titer may be taken as an indicia of progress with a vaccine, and vice versa. Hence, a further aspect of the invention is a method for monitoring efficacy of a vaccine, following administration thereof, by determining levels of antibodies in the subject which are specific for the vaccine itself, or a large molecule of which the vaccine is a part.

The identification of the subject proteins as being implicated in pathological conditions such as cancer also suggests a number of therapeutic approaches in addition to those discussed supra. The experiments set forth supra establish that antibodies are produced in response to expression of the protein. Hence, a further embodiment of the invention is the treatment of conditions which are characterized by aberrant or abnormal levels of one or more of the proteins, via administration of antibodies, such as humanized antibodies, antibody fragments, and so forth. These may be tagged or labelled with appropriate cytostatic or cytotoxic reagents.

T cells may also be administered. It is to be noted that the T cells may be elicited *in vitro* using immune responsive cells such as dendritic cells, lymphocytes, or any other immune responsive cells, and then reperfused into the subject being treated.

Note that the generation of T cells and/or antibodies can also be accomplished by administering cells, preferably treated to be rendered non-proliferative, which present relevant T cell or B cell epitopes for response, such as the epitopes discussed supra.

The therapeutic approaches may also include antisense therapies, wherein an antisense molecule, preferably from 10 to 100 nucleotides in length, is administered to the subject either “neat” or in a carrier, such as a liposome, to facilitate incorporation into a cell, followed by inhibition of expression of the protein. Such antisense sequences may also be incorporated into

appropriate vaccines, such as in viral vectors (e.g., Vaccinia), bacterial constructs, such as variants of the known BCG vaccine, and so forth.

Other features and applications of the invention will be clear to the skilled artisan, and need not be set forth herein. The terms and expression which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expression of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

We claim:

1. Isolated nucleic acid molecule which encodes a cancer associated antigen, whose amino acid sequence is identical to the amino acid sequence encoded by the nucleotide sequence of SEQ ID NO: 1, 3, 4, 8 or 15.
2. The isolated nucleic acid molecule of claim 1, comprising the nucleotide sequence of SEQ ID NO: 1.
3. The isolated nucleic acid molecule of claim 1, comprising the nucleotide sequence of SEQ ID NO: 3.
4. The isolated nucleic acid molecule of claim 1, comprising the nucleotide sequence of SEQ ID NO: 4.
5. The isolated nucleic acid molecule of claim 1, comprising the nucleotide sequence of SEQ ID NO: 8.
6. The isolated nucleic acid molecule of claim 1, comprising the nucleotide sequence of SEQ ID NO: 15.
7. Expression vector comprising the isolated nucleic acid molecule of claim 1, operably linked to a promoter.
8. Eukaryotic cell line or prokaryotic cell strain, transformed or transfected with the expression vector of claim 7.
9. Isolated cancer associated antigen comprising all or part of the amino acid sequence encoded by SEQ ID NO: 1, 3, 4, 8 or 15.
10. Eukaryotic cell line or prokaryotic cell strain, transformed or transfected with the isolated nucleic acid molecule of claim 1.

11. The eukaryotic cell line or prokaryotic cell strain of claim 10, wherein said cell line is also transfected with a nucleic acid molecule coding for a cytokine.

12. The eukaryotic cell line or prokaryotic cell strain of claim 11, wherein said cell line is further transfected by a nucleic acid molecule coding for an MHC molecule.

13. The eukaryotic cell line or prokaryotic cell strain of claim 11, wherein said cytokine is an interleukin.

14. The eukaryotic cell line or prokaryotic cell strain of claim 13, wherein said interleukin is IL-2, IL-4 or IL-12.

15. The eukaryotic cell line or prokaryotic cell strain of claim 10, wherein said cell line has been rendered non-proliferative.

16. The eukaryotic cell line of claim 10, wherein said cell line is a fibroblast cell line.

17. Expression vector comprising a mutated or attenuated virus and the isolated nucleic acid molecule of claim 1.

18. The expression vector of claim 17, wherein said virus is adenovirus or vaccinia virus.

19. The expression vector of claim 18, wherein said virus is vaccinia virus.

20. The expression vector of claim 18, wherein said virus is adenovirus.

21. Expression system useful in transfecting a cell, comprising (i) a first vector containing a nucleic acid molecule which codes for the isolated cancer associated antigen of claim 10 and (ii) a second vector selected from the group consisting of (a) a vector containing a nucleic acid molecule which codes for an MHC or HLA molecule which presents an antigen derived from said cancer associated antigen and (b) a vector containing a nucleic acid molecule which codes for an interleukin.

22. Immunogenic composition comprising the isolated cancer antigen of claim 9, and a pharmaceutically acceptable adjuvant.

23. The immunogenic composition of claim 22, wherein said adjuvant is a cytokine, a saponin, or GM-CSF.

24. Immunogenic composition comprising at least one peptide consisting of an amino acid sequence of from 8 to 12 amino acids concatenated to each other in the isolated cancer associated cancer antigen of claim 10, and a pharmaceutically acceptable adjuvant.

25. The immunogenic composition of claim 24, wherein said adjuvant is a saponin, a cytokine, or GM-CSF.

26. The immunogenic composition of claim 23, wherein said composition comprises a plurality of peptides which complex with a specific MHC molecule.

27. Immunogenic composition which comprises at least one expression vector which encodes a peptide derived from the amino acid sequence encoded by SEQ ID NO: 1, 3, 4, 8 or 15.

28. The immunogenic composition of claim 27, wherein said at least one expression vector codes for a plurality of peptides.

29. Vaccine useful in treating a subject afflicted with a cancerous condition comprising the isolated eukaryotic cell line of claim 10 and a pharmacologically acceptable adjuvant.

30. The vaccine of claim 29, wherein said eukaryotic cell line has been rendered non-proliferative.

31. The vaccine of claim 30, wherein said eukaryotic cell line is a human cell line.

32. A composition of matter useful in treating a cancerous condition comprising a non-proliferative cell line having expressed on its surface a peptide derived from the amino acid sequence encoded by SEQ ID NO: 1, 3, 4, 8 or 15.

33. The composition of matter of claim 32, wherein said cell line is a human cell line.

34. A composition of matter useful in treating a cancerous condition, comprising (i) a peptide derived from the amino acid sequence encoded by SEQ ID NO: 1, 3, 4, 8 or 15, (ii) an MHC or HLA molecule, and (iii) a pharmaceutically acceptable carrier.

35. Isolated antibody which is specific for the cancer antigen of claim 9.

36. The isolated antibody of claim 35, wherein said antibody is a monoclonal antibody.

37. Method for screening for cancer in a sample, comprising contacting said sample with a nucleic acid molecule which hybridizes to all or part of the molecule encoded by SEQ ID NO: 1, 2, 3, 4, 8 or 15 and determining hybridization as an indication of cancer cells in said sample.

38. A method for screening for cancer in a sample, comprising contacting said sample with the isolated antibody of claim 35, and determining binding of said antibody to a target as an indicator of cancer.

39. Method for diagnosing a cancerous condition in a subject, comprising contacting an immune reactive cell containing sample of said subject to a cell line transfected with the isolated nucleic acid molecule of claim 1, and determining interaction of said transfected cell line with said immunoreactive cell, said interaction being indicative of said cancer condition.

40. A method for determining regression, progression of onset of a cancerous condition comprising monitoring a sample from a patient with said cancerous condition for a

parameter selected from the group consisting of (i) a protein encoded by SEQ ID NO: 1, 2, 3, 4, 8 or 15, (ii) a peptide derived from said protein, (iii) cytolytic T cells specific for said peptide and an MHC molecule with which it non-covalently complexes, and (iv) antibodies specific for said CT protein, wherein amount of said parameter is indicative of progression or regression or onset of said cancerous condition.

41. The method of claim 40, wherein said sample is a body fluid or exudate.
42. The method of claim 40, wherein said sample is a tissue.
43. The method of claim 40, comprising contacting said sample with an antibody which specifically binds with said protein or peptide.
44. The method of claim 43, wherein said antibody is labelled with a radioactive label or an enzyme.
45. The method of claim 43, wherein said antibody is a monoclonal antibody.
46. The method of claim 40, comprising amplifying RNA which codes for said protein.
47. The method of claim 46, wherein said amplifying comprises carrying out polymerase chain reaction.
48. The method of claim 40, comprising contacting said sample with a nucleic acid molecule which specifically hybridizes to a nucleic acid molecule which codes for or expresses said protein.
49. The method of claim 49, wherein said nucleic acid molecule comprises SEQ ID NO: 9, 10, 11, 12, 13, 14, 17 or 18.
50. The method of claim 40, comprising assaying said sample for shed protein.

51. The method of claim 40, comprising assaying said sample for antibodies specific for said protein, by contacting said sample with protein.

52. Method for diagnosing a cancerous condition comprising assaying a sample taken from a subject for an immunoreactive cell specific for a peptide derived from a protein encoded by SEQ ID NO: 1, 2, 3, 4, 8 or 15, complexed to an MHC molecule, presence of said immunoreactive cell being indicative of said cancerous condition.

53. Composition comprising at least one peptide consisting of an amino acid sequence of from 8 to 25 amino acids concatenated to each other in the isolated cancer associated antigen of claim 9, and a pharmaceutically acceptable adjuvant.

54. The composition of claim 53, wherein said adjuvant is a saponin, a cytokine, or GM-CSF.

55. The composition of claim 53, comprising a plurality of MHC binding peptides.

56. Composition comprising an expression vector which encodes at least one peptide consisting of an amino acid sequence of from 8 to 25 amino acids concatenated to each other in the isolated cancer associated antigen of claim 9, and pharmaceutically acceptable adjuvant.

57. The composition of claim 56, wherein said expression vector encodes a plurality of peptides.

58. A method for screening for possible presence of a pathological condition, comprising assaying a sample from a patient believed to have a pathological condition for antibodies specific to at least one of the cancer associated antigens encoded by SEQ ID NOS: 1, 2, 3, 4, 8, or 15, presence of said antibodies being indicative of possible presence of said pathological condition.

59. The method of claim 58, wherein said pathological condition is cancer.

60. The method of claim 58, wherein said cancer is melanoma.
61. The method of claim 60, further comprising contacting said sample to purified cancer associated antigen encoded by SEQ ID NO: 1, 3, 4, 8, or 15.
62. A method for screening for possible presence of a pathological condition in a subject, comprising assaying a sample taken from said subject for expression of a nucleic acid molecule, the nucleotide sequence of which comprises SEQ ID NO: 1, 2, 3, 4, 8 or 15, expression of said nucleic acid molecule being indicative of possible presence of said pathological condition.
63. The method of claim 62, wherein said pathological condition is cancer.
64. The method of claim 62, comprising determining expression via polymerase chain reaction.
65. The method of claim 62, comprising determining expression by contacting said sample with at least one of SEQ ID NO: 9, 10, 11, 12, 13 or 14.
66. A method for determining regression, progression of onset of a cancerous condition comprising monitoring a sample from a patient with said cancerous condition for a parameter selected from the group consisting of (i) a cancer associated antigen encoded by SEQ ID NO: 1, 2, 3, 4, 8 or 15 (ii) a peptide derived from said cancer associated antigen, (iii) cytolytic T cells specific for said peptide and an MHC molecule with which it non-covalently complexes, and (iv) antibodies specific for said cancer associated antigen, wherein amount of said parameter is indicative of progression or regression or onset of said cancerous condition.
67. The method of claim 66, wherein said sample is a body fluid or exudate.
68. The method of claim 66, wherein said sample is a tissue.
69. The method of claim 66, comprising contacting said sample with an antibody which specifically binds with said protein or peptide.

70. The method of claim 69, wherein said antibody is labelled with a radioactive label or an enzyme.

71. The method of claim 69, wherein said antibody is a monoclonal antibody.

72. The method of claim 66, comprising amplifying RNA which codes for said protein.

73. The method of claim 72, wherein said amplifying comprises carrying out polymerase chain reaction.

74. The method of claim 66, comprising contacting said sample with a nucleic acid molecule which specifically hybridizes to a nucleic acid molecule which codes for or expresses said protein.

75. The method of claim 66, comprising assaying said sample for shed cancer associated antigen.

76. The method of claim 66, comprising assaying said sample for antibodies specific for said cancer associated antigen, by contacting said sample with said cancer associated antigen.

77. Method for screening for a cancerous condition comprising assaying a sample taken from a subject for an immunoreactive cell specific for a peptide derived from a cancer associated antigen encoded by SEQ ID NO: 1, 2, 3, 4, 8, or 15 complexed to an MHC molecule, presence of said immunoreactive cell being indicative of said cancerous condition.

78. An isolated nucleic acid molecule consisting of a nucleotide sequence defined by SEQ ID NO: 1, 2, 3, 8, or 15.

79. Isolated nucleic acid molecule the complimentary sequence of which hybridizes, under stringent conditions, to the nucleotide sequence set forth in SEQ ID NO: 4, 5, 8, or 15.

**ABSTRACT OF THE DISCLOSURE**

The invention relates to newly identified cancer associated antigens. It has been discovered that each of these molecules provokes antibodies when expressed by a subject. The ramifications of this observation are also a part of this invention.

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<i>ING2:</i>	AAAGCGTTCTGGCGGCAGCGCAACAACTAGAACCGTGAGAACCGTCCAGCAACCGGACCCACGACGTCA	75
<i>ING1 (D) :</i>	C.....C..A.....G.....A.....-.....G.G	
<i>ING2:</i>	CCTCGGGCACGCCAAGGAGAAGAAAGCCCAGACCTCTAAGAAGAACCGAGGGCTCCATGCCAAGCGTAGCGGC	150
<i>ING1 (D) :</i>	.....A.....G..A.....C.....A..C.....A.....G...AG	
<i>ING2:</i>	AGCGTCCCCCGACCTCCCATCGACCCAGCGAGCCCTCTACTGG-----G	201
<i>ING1 (D) :</i>	.....T..C.....A..A.G....TCTGTGCAACCAGGTCTCCTATGGG.	
<i>ING2:</i>	<u>AGATGATCCGCTGCGACAACGAA--TGCCCCATCGAGTGTTCCGTTCTCGTGTGAGTCTAACCATAAAC</u>	273
<i>ING1 (D) :</i>	.....G.....CGAG.....A.....C..G.G.....T.....	
<i>ING2:</i>	<u>CAAAGCGCAAGTGGTACTGTTCCAGATGCCGGGAAAGAACGA-----TGGGCAAAGCCCTTGAGAACGTCCA</u>	340
<i>ING1 (D) :</i>	.C...G.....C..AG.....GG.....GAAGACCA..A.....GGA..A....	
<i>ING2:</i>	GAAAAAAAACAGGGCTTATAACAGGTAGTTGGGACATCGCTTAATAGTGAGGAGAACAAAATAAGCCAGTGT	415
<i>ING1 (D) :</i>	--.....G.G.....C.....T.....G..C..GG.....G.....A.....	
<i>ING2:</i>	GTTGATTACATTGCCACCTTGCTGAGGTGCAGGAAGTGTAAAATGTATATTTTAAAGAATGTTGTTAGAGGCC	490
<i>ING1 (D) :</i>	A..T.....TG.....T.....A.G.....AGA.A...AA	
<i>ING2:</i>	GGGCGCGGTGGCTACGCCTGTAATCCCAGCACTTGGGAGGCCAGGCCGTCGGATCACGAGGTCAAGGAGATCG	565
<i>ING1 (D) :</i>	CCATT.CT.TCA.AGG.A-..GC.GTGATT.TG....CCTTTGTTT..AT.G.T.-....T.TAACAGAA.AGT	
<i>ING2:</i>	AGACCATCCTGGCTAACACGGTGAAACCCCGTCTCTACTAAAATTCAAAAAAAATTAGCTGGCGTGGTGGC	640
<i>ING1 (D) :</i>	G.T.TG.GGATCAGC.TTTA-....TA.AAA.A..GGTTG.....C.CTT..G.CTCAGACTGA.TTCTTG	
<i>ING2:</i>	GGGCGCTGTAGTCCCAGCTATTGGGAGGTGAGGCAGGAGAACGCTGGAGGTGGAGCTTGCANT	715
<i>ING1 (D) :</i>	C..GAGGAGGG.GA.T.AACTCA.CCT.ACACATTA.A.TGT.G.A..AAAAT.TTTCATTA.CTTTTA.ATTTTA	
<i>ING2:</i>	GAGCCAAGGTGCGGCCACTGCACTCCAGCCTGGCGACAGAGCGAGACTCCATCTTA	772
<i>ING1 (D) :</i>	ATA-....TAATATTATTACTTTATG.A.ATTTTTTTA.TT.GCCA.GTCG.CA.	

*ING2 = SEQ ID NO: 8*

*ING1(D) = SEQ ID NO: 2 (part)*

**SEQ ID NO. 15**

CTCGTCCGTTAAAGATGGTCTTCTGAAGGCTA**ACTGCGGA****ATGAAAGTTCTATTCCA**ACTAAAGCC.  
TTAGAATTGATGGACATGCAA**ACTTCAAAGCAGAGCCTCCGAGAAGCC**CATCTGCCTTCGAGCCTGC  
CATTGAAATGCAA**AAAGTCTGTTCAAATAAGCCTT**GAATTGAAGAATGAACAAACATTGAGAGCAG  
ATGAGATACTCCC**CATCAGAACAAACAAAGGACTATGAAGAAAGTCTT**GGATTCTGAGAGTCTC  
TGTGAGACTGTT**CACAGAAGGATGTGT**TTACCCAAAGGCTACACATCAAAAGAAATAGATAAAAT  
AAATGGAAAATTAGAAGAGT**CTCCTGATAATGATGGTTCTGAGGCTCCCTG**CAGAATGAAAGTT  
CTATTCCA**ACTAAAGCCTTAGAATTGATGGACATGCAA**ACTTCAAAGCAGAGCCTCCGAGAAGCCA  
TCTGCCTTCGAGCCTGCCATTGAATGCAA**AAAGTCTGTTCAAATAAGCCTT**GAATTGAAGAATGA  
ACAAACATTGAGAGCAGATCAGAT**GTTCCCTCAGAATCAAACAAAGAAGGTTGAAGAAAATTCTT**  
GGGATTCTGAGAGT**CTCCGTGAGACTGTT**CACAGAAGGATGTGTACCCAAAGGCTACACATCAA  
AAAGAAATGGATAAAATAAGTGGAAAATTAGAAGATTCAACTAGCCTATCAAAATCTGGATA**CAGT**  
TCATTCTGTGAAAGAGCAAGGGAA**CTTC**AAAAGATCACTGTGAACACGTACAGGAAA**ATGGAAC**  
AAATGAAAAAGAAGT**TTTGT**TACTGAAAAAGAA**ACTGT**CAGAAGC**AAAGAAATAACAGTTA**  
GAGAAC**AAAGTTAATGGGACAAGAGCTCTGAGATTGACTTAAACCAAGAAGAAGA**  
GAAGAGAAGAA**ATGCCGATATATTAAATGAAAAAATTAGGGAAAGAATTAGGAAGAATCGAAGAGCAGC**  
ATAGGAAAGAGTTAGAAGT**GAAACAAC**ACTTGAA**CAGGCTCTCAGAATACAAGATATAGAATTGAG**  
AGTGTAGAAAGTAATTGAA**TCAGGTTCTCACACTCATGAAAATGAAAATTATCTTACATGAAAAA**  
TTGCATGTTGAAAAGGAA**ATTGCCATGCTAAACTGGAAATAGCCACACTGAAACACCAATACCAGG**  
AAAAGGAAA**ATAATACTT**GAGGACATTAAGATT**TTAAAGAAAAGAATGCTGA**ACTTCAGATGACC  
CTAAA**ACTGAAAGAGGAATCATTA**ACTAA**AGGCATCTCA**ATATAGTGGGCAGCTAA**AGTTCTGAT**  
AGCTGAGAAC**ACAATGCTCACTCTAAATTGAAAGGAAAACAAGACAAAGAAATACTAGAGGCAGAAA**  
TTGAATC**ACACCACCTCTAGACTGGCTCTGCTGT**ACAAGACC**ATGATCAAATTGTGACATCAAGAAA**  
AGTCAAGAAC**CTGCTTCCACATTG**CAGGAGAT**GCTTGTGCAAAGAAAATGAATGTTGATGTGAG**  
TAGTACCGATATA**TAAC**AA**ATGAGGTGCTCCATCAACC**ACTTCTGAAG**CTCAAAGGAATCCANAAGC**  
CTAAA**ATTAACTCAATTATG**CAGGAGAT**GCTCTAAGAGAAAATACATTGGTTTCAGGAACATGCAC**  
AAAGAGAC**CAACGTGAAACAC**AGT**GTCAAATGAAGGAAGCTGAACACATGNTCAAANC**GAAC**AAGAT**  
NATGTGAAC**AAACACACTG**ANCAGCAGGAGT**CTCTAGATCAGAAATTATTCAACTACAAAGC**AAAAA  
TATGTGGCT**CAACAGCAATTAGTCATG**CACATAANGAAAG**CTGACAACAAAGCAAGATAACAATT**  
GATNT**TCATTNTCTTGAGAGGAAATGCNCATCATCTT**CAAAGAGAAA**ATGAGGAGATATTNAT**  
TACNATAACC**ATTAAAAACCGTATATTCAATATG**AAAAAAA**ANAAAAAAA**

**SEQ ID NO. 16**

MKVSIPTKALELMDMQTFKAEPPEKPSAFEPAIEMQKSVNKALELKNEQTLRADEILPSESKQKDYE  
ESSWDSESLCETVSQDVCLPKATHQEIDKINGKLEESPNDGFLKAPCRMVSIPTKALELMDMQT  
FKAEPPEKPSAFEPAIEMQKSVNKALELKNEQTLRADQMFPSESKQKKVEENSWDSESLRETVSQD  
VCVPKATHQE**MDKISGKLEDSTSLSKILD**TVHSCERARELQKDHEQRTGKMEQMKKFCVLKKL  
EAKEIKS**QLENQKVWEQELCSVR**LTLNQEEKRRNADILNEKIREELGRIEQHRKELEV**KQQLEQA**  
LRIQD**IELKSVESNLNQVS**HENENYLLHENCM**LKKEIAMLKLEIATLKH**HQYQEKENKY**FEDIKILK**  
EKNAEL**QMTLKLKEESLT**KRASQYS**QQLKVLIAENTMLTSKLKE**QDK**EAEIESHHPR**LASAVQD  
HDQIVTSRK**SQEPAFHIAGDACLQRKMNV**DVSSTD**I**.

*SEQ ID NO: 2*

GAGTAACCGATAATATGCCGTTGTGCACGGCAGAGAATTCCAGATATAGCAGTAGCAGTGATCCCAGGCCT  
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GCATCTTGCAGTCGGACAGAGACAGACGGCGAGATGCTGCACTGTGTGCAGCGCGCCTGATCCGC  
GAGCGCTTCAGTCGAGACAGACGGCGAGATGCTGAGCCAGATGGTGGAGCTGGTGGAGAACCGOACGCAG  
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GTGGACAGGCCACGTGGAGCTGTCAGGCAGCAGGAGCTGGCGACACAGTGGCAACAGCGGCAAGGTTGGC  
CGGGACAGGCCAATGGCGATGGCTAGCGCAGTCTGACAAGGCCAACAGCAAGCGCTACGGCGCAGCGAAC  
AACAGAAACCGTGGAGAACCGCTCAGCAACCACGACCACGACGACGGCGCCTCGGGCACACCCAAGGAGAAC  
GCCAAGACCTCCAAGAAGAACGCTCCAAGGCCAAGGCCAGGGAGCGAGAGGGCTCCCTGCCACCTCCCCATC  
GACCCCAACGAACCAACGACTGTCTGCAACCAGGTCTCCTATGGGAGATGATCGGCTGCACACGACGAG  
TGCCCCCATCGAGTGGTCCACTCTCGCTGCGTGGGCTCAATCATAAACCAAGGGCAAGTGGTACTGTCCCAAG  
TGCCGGGGGAGAACGAGAACGACATGGACAAGGCCCTGGAGAAATCCAAAAAGAGAGGGCTTACACAGGTAG  
TTTGTGGACAGGCCCTGGTGTGAGGAGGACAAAATAACCGTGTATTATACATTGCTGCCTTGTGAGGTG  
CAAGGAGTGTAAAATGATATTTAAAGAATGTTAGAAAAGGAACCATTCCCTTCATAGGGATGGCAGTGATTC  
TGTTTGCCTTTGTTCATGGTACACGTGTAACAAGAAAGTGGTCTGTGGATCAGCATTTAGAAAATACAAA  
TATAGGTTGATTCAACACTTAAGTCTCAGACTGATTCTTGCAGGAGGAGGGGACTAAACTCACCTAACACA  
TTAAATGTGGAAGGAAAATATTCATTAGCTTTTATTTAATACAGTAATATTACTTATGAACAATT  
TTTTAATGGCCATGCGCAAAATACAGCCTATAGTAATGTGTTCTGCTGCCATGATGTATATCCATAT  
AACAAATTCAGTAACAAAGGTTAAAGTTGAAGATTATTTTAAAGGTTAAAGGTTAAATTTACATGACAG  
ATATTTATCTATTGGCTGTCCTCCAAATGGCATTAAATGCTGGGTACACTCTCTTAAGTGGTCTAGT  
CAAGGAACCTCAAGTCATGCTTGTCTACCAATCATAGTGTACCCATCTTAATTATATCAGGTGTATAAA  
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TCACAATTATGTGCAAAGGTGTGCTCTGCTGTATGTGAGCTGTAAAATGTTACGTGAAGAAATAATGAAAC  
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ACACCAGCAATTAGACAAAGCCTAACGAAATTGTGTTATTGCTACTTATTATAATAATGAAGTAGAA  
GTTACTTAATTGCCAGCAAATAACGTGCAAAAAAGAATCTGTATTCAAGACCCCTGGGTCAAGGAAATTACT  
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GTTTCAGCGGCACCCAAAAAA

gi|2183221|gb|AAB60879.1| p33ING1 (*SEQ ID NO: 12*)

MPLCTATRI PRYSSSDPGPVARGRGCSSDRLPRPAGPARQFQAASLLTRGWGRAWPWKQILKELDECY  
ERFSRET DGAQKRRMLHCVQRALIRS QELGDEKIQIVSQMVELVENRTRQVD SHVELFEAQ QELGDTVGN  
SGKVGADRPNGDAVAQSDKPN SKRSRRQRNNENRENASSNHDHDDGASGTPKEKKAKTSKKKRSKAKAE  
REASPADLPIDPNEPTYCLCNQVS YGEMIGCDNDECPIEWFH FSCVGLNH KPKGKWYCPKCRGENEKTMD  
KALEKS KKERAYNR

D:	MPLCTATRIPRYSSS	15
A:	MLSPANGEQLHLVNYVEDYLDIESLPFDLQRNVSLMREIDAKYQEILKELDECYERFSRET DGAQKRRMLHCVQ	75
B:	MLHCVQ	6
C:	MEILKELDECYERFSRET DGAQKRRMLHCVQ	31
D:	SDPGPVARGRGCSSDRLPRPAGPARQFQAASLLTRGWRAPWKQILKELDECYERFSRET DGAQKRRMLHCVQ	90
A:	RALIRSQELGDEKIQIVSQMVELVENRTQVDSHVELFEAQQELGDTVGNSKGKVGADRPNGDAVAQSDKPNSKRS	150
B:	RALIRSQELGDEKIQIVSQMVELVENRTQVDSHVELFEAQQELGDTVGNSKGKVGADRPNGDAVAQSDKPNSKRS	156
C:	RALIRSQELGDEKIQIVSQMVELVENRTQVDSHVELFEAQQELGDTVGNSKGKVGADRPNGDAVAQSDKPNSKRS	106
D:	RALIRSQELGDEKIQIVSQMVELVENRTQVDSHVELFEAQQELGDTVGNSKGKVGADRPNGDAVAQSDKPNSKRS	165
A:	RRQRNNNENRENASSNHDHDDGASGTPKEKKAKTSKKKRSKAKAEREASPADLPIDPNEPTYCLCNQVSYGEMIG	225
B:	RRQRNNNENRENASSNHDHDDGASGTPKEKKAKTSKKKRSKAKAEREASPADLPIDPNEPTYCLCNQVSYGEMIG	231
C:	RRQRNNNENRENASSNHDHDDGASGTPKEKKAKTSKKKRSKAKAEREASPADLPIDPNEPTYCLCNQVSYGEMIG	181
D:	RRQRNNNENRENASSNHDHDDGASGTPKEKKAKTSKKKRSKAKAEREASPADLPIDPNEPTYCLCNQVSYGEMIG	240
A:	CDNDECPIEFHFSCVGLNHKPKGWCYCPKCRGENEKTMDKALESKKERAYNR.	279
B:	CDNDECPIEFHFSCVGLNHKPKGWCYCPKCRGENEKTMDKALESKKERAYNR.	210
C:	CDNDECPIEFHFSCVGLNHKPKGWCYCPKCRGENEKTMDKALESKKERAYNR.	235
D:	CDNDECPIEFHFSCVGLNHKPKGWCYCPKCRGENEKTMDKALESKKERAYNR.	294

A = SEQ ID NO: 5

B = SEQ ID NO: 6

C = SEQ ID NO: 7

D = SEQ ID NO: 17

## COMBINED DECLARATION AND POWER OF ATTORNEY

Attorney Docket No.  
LUD 5615 (09905230)

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled "**ISOLATED NUCLEIC ACID MOLECULES ENCODING CANCER ASSOCIATED ANTIGENS, THE ANTIGENS PER SE, AND USES THEREOF**

", the specification of which

(check one)  is attached hereto.

was filed on  
and was amended on

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed:

## Prior Foreign Application(s)

			Priority Claimed	
(Number)	(Country)	(Day/Month/Year Filed)	<input type="checkbox"/>	<input type="checkbox"/>
			Yes	No
			<input type="checkbox"/>	<input type="checkbox"/>
			Yes	No
			<input type="checkbox"/>	<input type="checkbox"/>
			Yes	No

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below>

(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under Title 35, United States Code § 120 of any United States application(s) or § 365(b) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior U.S. or PCT international application in the manner provided by the first paragraph of Title 35, U.S.C. § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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